Detailed Product Review

BLA ref. number: 98-0012

Trade name: Remicade (provisional)

Manufacturer: Centocor

USAN Name: Infliximab

IND name: cA2

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Summary Description: Infliximab (cA2) is a chimeric anti-TNFα mAb. It is produced by [redacted] cells transfected with genomic expression constructs containing [redacted]VDJ_H and VJ_κ exons and human γ1 and κ exons. It is purified to near homogeneity by standard chromatography technology and will be marketed in a lyophilized form. It has a high affinity (Ka = 10^{10} M^{-1}) for TNFα and is specific for human and chimp TNF. The proposed indication is for parenteral use in Crohn's disease patients, with the mechanism of action believed to be clearance of pro-inflammatory TNF and possibly deletion of aberrantly activated $mTNF^+$ T cells.

Overall Conclusions and Recommendations: In general, the manufacturing process of cA2 at Centocor BV is well controlled. The specifications for purity and potency are tight and the consistency lots within specifications. Drug product manufacture at [redacted] is also controlled. In general, most manufacturing issues were resolved during the IND stage. All product issues raised in the review of the BLA have been resolved (see amendments) or phase IV commitments for their resolution are in place.

Reviewer's note: Reviewers notes are **bolded**, action items are listed at end. Where there is no reviewer's note the process and testing is deemed to be acceptable by the reviewer. Adobe photoshop scans integrated in text are stamped with BLA number and volume.

Detailed review table of contents:

Abbreviations: DPC, direct product capture; PFB, purified bulk; FLP, final lyophilized product; DSP, downstream processing.

DRUG SUBSTANCE

1. Description And Characterization

Reviewer's note: cA2 has been comprehensively characterized biochemically.

Description cA2 is a chimeric IgG1:κ monoclonal antibody specific for human and chimp TNFα. Like all IgG1 molecules it has a molecular weight of about 149 kD and is comprised of two identical heavy and light chains which associate by disulfide bonds and non-covalent interactions. It has 5 major glycoforms with N-linked asialo-,core-fucosylated, biantennary structures with terminal galactose microheterogeneity. Three charge isoforms exist, reflecting heterogeneity of C-terminal lysine content.

Characterization / Proof Of Structure

Physicochemical Characterization of Reference Standard

Characterization focused on reference standard FLP lot 96H06 and clinical FLP lot 95K06. These lots derived from the same downstream processing (DSP) lot, 5J005. The characterization included:

- Molecular modeling. The predicted structure of cA2 was determined by homology modeling using the cA2 amino acid sequence and PDB structures. The structure of Fab fragments was generated first and then the structure of the whole molecule was generated. The Fab structure is probably more accurate because of the abundance of Fab fragments in the PDB.
- Mass spectroscopy. The molecular weights of the isoforms of cA2 (lot 96H06) range from This is consistent with the predicted cA2 amino acid composition and glycoforms with 0-4 galactoses. When treated with carboxypeptidase C, there was a reduction in heterogeneity and the isoforms ranged from Analytical ultracentrifugation confirmed the molecular weight measurement
- N-terminal sequencing of SDS-PAGE purified heavy and light chain (lot 95K06). The size of bands seen in reduced and non-reduced SDS-PAGE gels were consistent with IgG1 and the IgG1 sub-unit structure. The N-terminal sequences generated from excised bands were consistent with the predicted amino acid sequence of cA2. A assay found that cA2 has 0.53% free sulfhydryls.
- Tryptic mapping and mass spec (lot 96H06) fragments result from trypsin digestion. They were able to identify each based on the predicted molecular weight of tryptic fragments of cA2 and on the mass spectroscopy data.

- Glycoform analysis (lot 5J005). Two possible N-linked glycosylation sites on cA2 were identified based on the predicted amino acid sequence: Mass spectroscopy analysis of the light chain reveals that Monosaccharide composition and oligo saccharide mapping data reveals that the three oligosaccharides are No O-linked glycosylation was detected.
- Charge microheterogeneity. Three predominant charge isoforms are detectable by IEF and ion exchange chromatography (IEC): The three charge isoforms were still apparent after carboxypeptidase treatment, arguing that the isoforms do not reflect differences in glycosylation. C-terminal amino acid sequencing of each isoform isolated by IEC identified them containing 0, 1, or 2 heavy chain C-terminal lysines. C-terminal lysine heterogeneity is a common feature of proteins expressed in mammalian tissue culture. In lot 96H06, of the heavy chains contained C-terminal lysine. The C-terminal lysine heterogeneity was confirmed by analysis of tryptic fragments. N-terminal sequencing of the IEC purified isoforms revealed no heterogeneity of N-terminal amino acid sequences. Minor isoforms were identified as product degradents in the impurities profile.
- Circular dichroism. The CD spectrum of cA2 was consistent with that of a properly folded antibody, e.g. the secondary structure is mostly β-sheet. The far UV CD spectrum was consistent with the high Tyr and Trp content of the variable regions.
- Analytical ultracentrifugation (lot 96H06). The self-association tendency of cA2 was measured by analytical ultracentrifugation. The K_a of the monomer to dimer reaction is The K_a is about 10 fold lower in 10% sucrose (formulation buffer). Antibodies have a natural tendency to aggregate.
- Extinction coefficient. The extinction coefficient of cA2 was measured by gravatometric and spectroscopic analysis.

Qualifying Lots

There are 5 qualifying lots. **Reviewer's note: the 5 qualifying lots demonstrate consistant manufacture of drug substance.**

DSP number	Scale	Formulation	FLP lot numbers
5J005		Lyo I	95K06
			96E06
Z6M022		Lyo II	97A07
			97A10
Z7A286		Lyo II	97C07
Z7D117		Lyo II	97E08
			97E09
Z7H382		Lyo II	97K10

- Potency. The bioassay revealed that the average potency of these lots is 95.9 +/- 10.5%. **Note: Results from lot Z7H382 are pending** . No trend of increased or decreased potency was apparent between scale lots.
- Purity. Purity by SDS-PAGE is >98.0% for each lot (average 98.5 +/- 0.4%). Purity by GF-HPLC is >99.9% monomer for each lot. **Note: Results from lot Z7H382 are pending (** . No trend of increased or decreased purity was apparent between scale lots. **Note:**
- N-terminal sequencing (identity). N-terminal sequencing of each lot is consistent with the known cA2 sequence.
- Tryptic mapping (identity). Tryptic fragments of each lot were analyzed by reverse phase HPLC and mass spectroscopy. The tryptic fragment patterns were identical between lots with a few exceptions. The peak eluting at identified as of the heavy chain was variable between lots. The variable intensity was identified as Variability of the relative order of peaks eluting between was identified as charge state heterogeneity.
- Circular dichroism (secondary and tertiary structure identity). CD spectra were equivalent between lots.
- C-terminal lysine. The percentages of heavy chains with C-terminal lysine ranged from No trends of higher or lower percentages of C-terminal lysine were evident between scale lots.
- IEF. predominant bands are present in IEF patterns from each lot. Each lot scored >97.6% in the IEF similarity index.
- Cation exchange chromatography. Each lot scored >98.0% in the cation exchange chromatography similarity index except lot Z7H382 with a score of 93.6%. The lower value reflected a decreased level of minor isoforms with pI values

- Oligosaccharide analysis. The percentages of agalacto, monogalacto and digalacto oligosaccharide structures were measure for each lot by [redacted] from each lot. Agalacto oligosaccharides ranged from [redacted] monogalacto oligosaccharides ranged from [redacted] and digalacto oligosaccharides ranged from [redacted] of total oligosaccharides. No trends were evident between lots.

2. Manufacturer(s)

Identification:

- Centocor B.V., Leiden, The Netherlands (drug substance)
- [redacted] letter present)
- [redacted] contract QA testing, master file [redacted] cross-reference letter present)

Floor Diagram- Diagrams of campus layout, air classification, personnel flow, material & equipment flow, and product flow of both upper and ground floor are present in volume 3.

Other Products: Panorex, ReoPro, Myoscint.

3. Method(s) Of Manufacture

Reviewer's note: In general, the method of manufacture is adequate. The cell bank safety characterization is complete and virus removal validation studies have been performed. The process has an adequate number of chromatography steps to ensure high purity product. Issues raised during the review have been addressed in subsequent amendments.

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Raw Material	Monograph[a]	Manufacturing Process

[redacted]

[a] NCT = Not Compendially Tested; NF = National Formulary; NCL = Not Compendially Listed; EP = European Pharmacopoeia; USP = United States Pharmacopoeia

[b] Except for degree of hydration

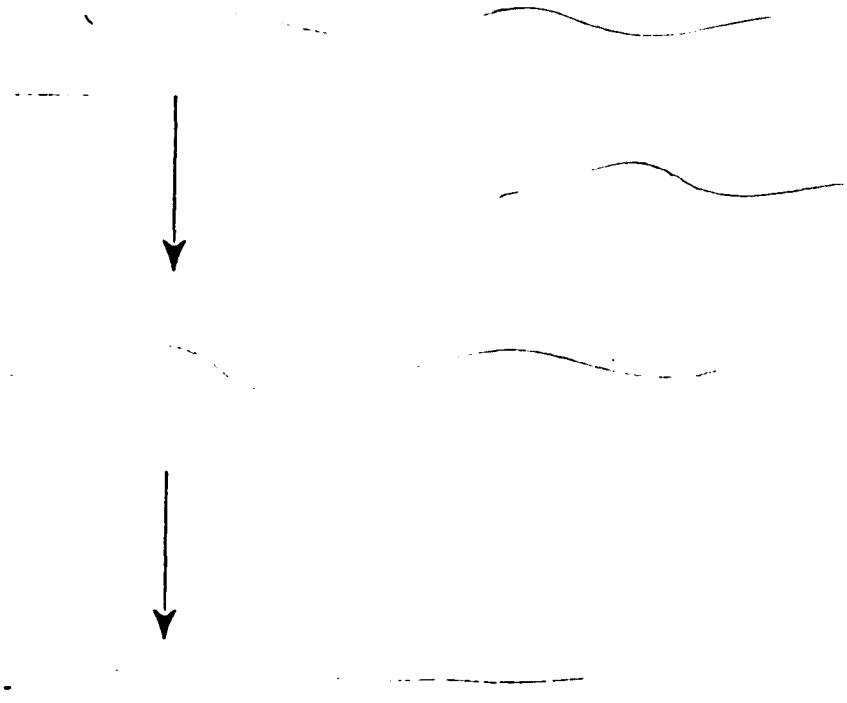
Raw materials are mostly Compendial, with the exceptions of some chemical and bovine media components, column resins and some chemicals

[redacted]

[redacted] is a porcine pepsin lysate of beef protein. Certificate of Analysis are supplied for each animal source product. Source herds for these products are from USA or [redacted]). Each bovine product (except [redacted] and [redacted]) are tested by their manufacturer for bacteria/fungi, mycoplasma and bovine viruses. Centocor inspects them for color, appearance, growth promotion and identity/protein content. [redacted] can contain up to [redacted] ppm bovine IgG. The [redacted] C of A for [redacted] does not list any specific adventitious agent test but states that "source animals received ante- and post mortem inspections under the supervision of a veterinarian" and the product was held at a pH <5.0 and/or temperature >55°C NLT 3 hr. The [redacted] C of A for [redacted] does not list any microbial tests beyond testing for coliforms and Salmonella. **Note: There is no testing for porcine parvovirus** . .

All non-Compendial chemicals are tested by Centocor for appearance, color, and identity. Other attributes like bioburden, pH and endotoxins are tested when appropriate . Pre-mixed media formulations like [redacted] and [redacted] supplement are tested for growth promotion, color, appearance, pH and osmolality. Other attributes like bioburden, endotoxins, solubility, glucose and amino acids are tested when appropriate. Column resins are tested for bioburden, appearance, color, identity and a functional attribute; [redacted] is tested for binding capacity while [redacted] are tested for particle size.

Flow Chart: The overall scheme of production is below. The solvent/detergent step is considered to be a division between "crude" and "refined" operations. Steps that occur before and after S/D are physically separated in the plant and performed by separate personnel.



Cell Substrate / Host Cell / Expression Vector System

Host Cells The transfection substrate for cA2 expression is the

, research bank in —— and a
—— on 10/90; the —— was found free of bacteria/fungi, mycoplasma, thymic

agent virus, bovine viruses and adventitious viruses (MAP; in vitro co-cultivation; in vivo inoculation into adult and suckling mice, guinea pigs and hen's eggs). . detected the production of retrovirus The origin of the was demonstrated by isozyme and karyology analysis. Cells from the research bank were used for transfection.

Gene Constructs

The cA2 chimeric mouse/human expression constructs were created using rearranged variable region genes from an anti-TNF hybridoma, A2. A2 was created by using standard hybridoma technology. BALB/c mice were initially immunized with

Genomic DNA was isolated from

and characterized by restriction mapping and sequencing.

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A

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Cloning of the Recombinant Cell Lines

is the cA2 producing cell substrate.

Master Cell Bank (MCB) & Working Cell Bank (WCB)

A [redacted], a [redacted] vial WCB ([redacted], and a [redacted] vial back-up WCB [redacted]) were established using cells grown in serum containing [redacted] media by controlled freezing them in [redacted] containing [redacted]. They are stored in LN_2 <-120°C. The [redacted] vials are stored in [redacted]. The main WCB is stored in [redacted] rest used for QC testing or production. The back-up WCB is stored in [redacted] rest used for QC testing. The MCB and WCBs have been tested for microbial agents in conformance with the mAb PTC, 1997 and the cell lines PTC, 1993:

Test	*20MAY91*	*WCB S91J134*	*WCB Z7E072*	*D83 EPC Z6L035*
Viability during preparation				
Viability of frozen bank				
Mycoplasma (PTC 1987)				
Cultivable	neg.	neg.	neg.	neg.
Co-cultivation with Vero	neg.	neg.	neg.	neg.
Sterility (21 CFR 610.12/USP)	neg.	neg.	neg.	neg.
MAP (LCM, Ectromelia, GDVII, LCM, Hantaan, MVM, MAV MHV, PVM, Polyoma, Reo-3, Sendai, EDIM, MCMV, K, Thymic, & LDHV	neg.	neg.	neg.	neg.
Thymic agent virus	neg.	neg.	neg.	neg.
Bovine viruses (BVD, IBR, PI3, BPV, BAV-3)	neg.	neg.	neg.	neg.
Extended S^+L^- focus	neg.	neg.	neg.	neg.
Extended XC plaque	neg.	neg.	neg.	neg.
Mus dunni (ERV)	neg.	**10 sfu/ml**	**5 sfu/ml**	neg.
Extended Mus dunni	**pos.**	n.d.	**pos.**	neg.
Dunni cell Co-culture (if Dunni is neg.)	**pos.**	**pos.**	n.d.	**pos.**
Extended MCF (Mink)	neg.	neg.	neg.	neg.
Extended MCF (SC-1)	neg.	neg.	neg.	neg.
TEM	**A & C**	**A & C**	**A & C**	**A & C**
RT	neg.	neg.	neg.	neg.

In vitro adventitious agents (HeLa, H9, MRC-5, Vero & C168J cells)	neg.	neg.	neg.	neg.
In vivo (suckling & adult mice, guinea pigs, embryonated hen eggs)	neg.	neg.	neg.	neg.
Karyology	murine	murine	murine	murine
Isozymes	murine	murine	murine	murine
Clonality of IgG production	100%	99%	100%	97%
Identity of secreted mAb (DRID)	reacts	reacts	reacts	reacts
Cell identity (idiotype FACS)	reacts	reacts	reacts	reacts
Stability of IgG production	100 generations	N.D.	N.D.	97% @ D 83
PBL co-cultivation (viral)	neg.	N.D.	N.D.	N.D.
PCR (HIV 1& 2, HTLV I)	neg.	N.D.	N.D.	N.D.
Caprine (Goat) CAV, CHV, CAEV virus	neg.	N.D.	N.D.	N.D.
DNA hybridization (HIV-1 & 2, HTLV-I & II, HBLV, EBV, CMV, JCV, HBV, BIV, BLV, SIV, SRV)	neg.	N.D.	N.D.	N.D.

Sterility, DNA hybridization, clonality, DRID, and FACS were performed by Tests that were performed by contract testing labs are:

- MAP, Thymic agent virus, in vivo adventitious virus, MCF virus, bovine viruses, PBL co-cultivation, HIV & HTLV PCR,
- Mycoplasma, in vitro adventitious virus, XC plaque, S+L- focus, Dunni cell, RT
- : TEM
- Caprine viruses
- : Karyology, Isozymes

End Of Production Cells (EPC)

End of production cells were collected at day of cA2 bioreactor lot Z6L035 on Day represents days of culture beyond the maximum cA2 bioreactor run, days. EPC cells, cell lysates, and supernatants were subjected to the same microbial tests as those routinely performed on WCBs (table above). No adventitious agents beyond the expected type A & C

retroviruses were detected in the EPC, and no induction of increased infectious retrovirus levels by production culture was seen.

Cell Growth And Harvesting

Three separate media formulations are used to culture C168J.

-
-
-

Pre-culture is initiated by thawing of WCB and seeding the cells into a T-75. Pre-culture expansion lasts The cells are maintained in for the rest of pre-culture. The cells are expanded in larger T/C flasks, and spinner baskets at seeding densities of When spinner basket cell densities allow, the cells are transferred to a seeding bioreactor at The bioreactor is monitored for DO_2, temp., pH, motor speed, cell density and viability and bioburden. Controls and specification for pre-culture and expansion are below:

Parameter	Set Point	Range
Temperature	36.5°C	
Dissolved oxygen (DO)	40%	
pH	7.3	
Motor speed (Spin filter + Impeller rpm)	75	

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Sample	Test	Specification
Preculture, diluted culture from	Cell population density (seeding density)	
Preculture sampling at every passage, days	Microscopic examination	
	Cell population density	
	Cell viability	
Preculture, inoculum	Microscopic examination	
	Cell viability	
	Cell population density	
	Total volume	
	Bioburden	
Sampling of inoculum fermenter, at inoculation	Cell population density	
Sampling of inoculum fermenter, sampled at least once per	Microscopic examination	
	Cell population density	
	Cell viability	
	Bioburden	

[a] In-process test methods are described in Section 2.1.4.1.
[b] The operation is terminated if

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When adequately expanded, of inoclulum culture is transferred to spin filter bioreactor at cells/ml. One bioreactor may inoculate more than one bioreactors. The cells are allowed to expand by increasing the rate and the volume of culture to When cell density reaches cells/ml, the media is switched to cells/ml The bioreactor culture is monitored for DO_2, temp., pH, motor speed, spin filter speed, cell viability and density, bioburden and IgG content. Fermentation is terminated if test results for bioburden, IgG content, or

cell viability are outside of specifications, or if the culture length from inoculation of — . fermenter has exceeded —days. Harvests are clarified by in-line filtration and stored in — polyethylene bags at —— for no more than days. Harvests are tested for adventitious agents in conformance with the mAb PTC, 1997. Testing and specifications for production are below:

Parameter	Set Point	Range
Temperature	36.5°C	——
Dissolved oxygen (DO)	40%	——
		——
pH	7.3	——
Motor Speed (rpm)	75	——
Spin filter speed (rpm)	85	——

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Sample	Test[a]	Specification
Fermenter sampling, at inoculation	Cell population density	
Fermenter sampling, at least once per 24 hours	Cell viability	———
	Microscopic examination	
	Bioburden	
	IgG content by nephelometry	———
	Cell population density	
Fermenter sampling at the end of fermentation	Mycoplasma Non-cultivable Cultivable	
	Retrovirus testing [d] Dunni Cell assay (direct) S^+L^- (direct) XC plaque assay (direct)	———
	In vitro test for adventitious viruses on: Vero cells MRC-5 cells HeLa cells Host C168J cells	———
Every harvest after disconnection from fermenter	pH Bioburden Endotoxin Microscopic examination	———

[a] In-process test methods are described in Section 2.1.4.1.

[b] If viability is below 70% for three consecutive days the fermenter run will be terminated.

[c] If the cA2 IgG concentration is below 50 μg/mL during the C
→ the fermenter run will be terminated.

[d] Murine retroviruses will be measured on the first 3 fermentation runs when a new Manufacturer's Working Cell Bank (MWCB) is instituted. If no virus is detected, subsequent fermenter runs derived from the same MWCB will not be tested. If virus is detected, testing will continue with the indicated specifications.

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Genetic Stability

Genetic stability studies were performed on the MCB, both WCBs, EPC cells (Z6L035 day 83), and production cells (PC) from three lots Z7B323 (day ~), 5D229 (day ~), and Z6L035 (days ———). Cells were examined for doubling time, Ig production, % Ig producers (ELISA), isozyme/karyology, and κ and γ1 gene gross structure (Southern blot), copy number, sequence stability

(sequencing of RT/PCR products), and expression (Northern blot). cA2 bioreactor runs do not exceed

Doubling time (——), Ig production (——, and % Ig producers (>97%) remained stable in a generation (——) scaled down study. Isozyme and karyology patterns in cells from the small scale study remained stable. Southern blot analysis of — WCB, PC and EPC revealed that no gross changes in κ and γ1 gene structure like deletions or insertions occurred. Band intensity in EPC was lighter, suggesting a reduction of copy number occurred over time. Sequencing of RT/PCR products from these cells did not reveal any aberrant sequences in these cells. **Note: the level of detection of this assay was 25% minority sequence** (——. Southern blotting of internal restriction fragments (Eco RI) with comparison to standards revealed that

t. An equivalent copy number was present in the —— WCBs, and PCs through day but only —heavy chain construct and ~ 8 light chain construct was present in the day EPC. As bioreactor runs are limited to days, copy number held steady during the length of a production run. Measurement of RNA levels revealed that γ1 and κ expression was higher in day —— and C than — or WCB cell. Expression at the level present in cell bank cells was seen in EPC. Thus, if anything, the production culture conditions up-regulate expression of Ig mRNA.

Purification And Downstream Processing

The first step in purification is direct product capture (DPC) of cA2 from raw harvests using columns. The harvest is applied at to the pre-equilibrated column . The column is washed with the pH —) tris buffer until the A_{280} of the effluent reaches baseline and then with irrelevant protein contaminants.

Stability studies of concentrated DPC eluate are on-going. In process testing and controls are below:

Sample	Test[a]	Specification
Harvests	Number of source fermenters	
Protein A column effluent, prior to loading	pH	
Ultrafiltration device filtrate after concentration	OD_{280}	
Concentrated cA2 IgG, prior to freezing	pH Osmolality Endotoxin Bioburden Identity by IEF Concentration by OD_{280} Purity by GF-HPLC	

[a] In-process methods are described in Section 2.1.4.1.
[b] Tested prior to final filtration of Protein A-purified cA2 IgG. May be adjusted with
[c] Tested prior to final filtration of Protein A-purified cA2 IgG. May be adjusted with

Next is a solvent/detergent virus inactivation step.

incubation tank for The incubation is terminated immediately by the subsequent cation exchange chromatography step. In process testing and controls for the thawing and pooling are below:

Sample	Test[a]	Specification
cA2 at the start of thawing	Total mass cA2 IgG	
Pooled eluate	Bioburden Endotoxin	
Pooled DPC cA2 IgG, post titration	pH	

[a] In-process test methods are described in Section 2.1.4.1.
[b] pH is adjusted by gradual addition of and may also be adjusted with Tris, untitrated.

The next step is a

Sample	Test[a]	Specification
Solvent/Detergent treated cA2 IgG, before dilution	pH	
column effluent prior to loading	Conductivity pH	
cA2 IgG during in-line dilution/loading	pH Conductivity	
Cation exchange purified cA2 IgG	Endotoxin Purity by GF-HPLC	

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The next step is

Tests and specifications are below:

Sample	Test[a]	Specification
Virus removal filter assembly	Endotoxin Bioburden	
Ultrafiltration device retentate/ filtrate lines prior to product processing	Endotoxin pH	
cA2 IgG during virus removal ultrafiltration	Concentration by OD_{280}	
cA2 IgG post virus removal ultrafiltration and concentration	Concentration by OD_{280}	

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Sample	Test[a]	Specification
Ultrafiltration device retentate and filtrate line effluent prior to product processing	Endotoxin pH	
cA2 IgG prior to final 0.2 μm filtration	Conductivity	
cA2 IgG preformulated bulk material prior to freezing	Endotoxin Bioburden Concentration by OD_{280} pH Bioactivity by WEHI assay	
	Purity by SDS-PAGE, reduced	
	Purity by SDS-PAGE, non-reduced	
	Purity and identity by GF-HPLC	

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Batch Records

A sample batch record was present for FLP lot 97E07 (bulk Z7D117, labeled 97E08AA & 97E09AA) filled and lyophilized at [redacted] (5/27-6/13 1997) and bulk lot Z7H382 [redacted] scale) manufactured at Centocor (2/12-9/23 1997). **Note: The batch records are very detailed and clear, but contained minor inconsistancies. These inconsistencies were resolved in**

Ask for a clarification lot nomenclature (question
. The batch record included:

- Preculture records of lots Z7A292, Z7D425, and Z7F378. Preculture lot Z7D425 (May 1997) was judged to be "questionable" by two different operators early in the expansion phase (day) They were tested for bioburden and found to be negative.
- Large scale culture records of bioreactor lots Z7A294, and Z7B323, Z7B324, and Z7F382.

- DPC records for lots Z7C044, Z7D053, Z7D055, Z7D056, Z7F012, Z7F014, Z7F015, Z7F016, Z7F017, Z7G254, Z7G255, Z7G256, Z7G257, Z7H197, were provided.

Note: ask why purity is listed as 100% at step when there are other peaks in the HPLC traces, ex. lot Z7G256, Z7C044, Z7D053 (
- Thawing and pooling records for lot Z7H382 were provided. 14 DPC lots were thawed and pooled to yield of cA2 in 160 L.
- Solvent/detergent and cation exchange records for lot Z7H382 were provided. After the cation exchange column the lot is divided into parts. Yield was about .
- records were provided for lots Z7H382-1 & 2. Nine cartridges were used for each part. Yield was
- Records for the first anion exchange column for lot Z7H382 were provided. The flow through (about cA2) was collected, yield
- Records for the second anion exchange column for lot Z7H382 were provided. The major peak and the first fraction were collected, the strip, flow through and subsequent peaks were discarded because they didn't pass specifications for aggregates. Yield was .
- Records for preformulation of Z7H382 were present. The final yield was kg.
- Records for freezing of preformulated Z7H382 were present. The pre-formulated bulk was frozen in separate containers (ach) at
- Records from of thawing, pooling and diluting of preformulated bulk; filtration of formulated bulk; preparation of formulation buffer; filling; lyophilization and capping. is the form attached to equipment used in operations and contain records of previous use (maintain dedicated status of equipment). In some instances it is hard to read these forms because they didn't use autoclave proof ink. In other cases, it isn't clear what equipment is dedicated: a "short needle bar (1.2)" previously was used to process); a "transfer hose" and a "20 L bottle" was

used to process [illegible], and a "res head" was used to process [illegible]. **Note: we should get a breakdown on what equipment is dedicated to cA2 and what is not (———).** Also, some environmental monitoring data is questionable: RODAC readings on 5-30-97 from floor A of room — had 18 cfu, but was "satisfactory" (action limit is — cfu/plate). Personnel readings from VAT's right hand on 6-5-97 were 28 cfu, but was scored as "pass" [illegible]). **Note: they should be asked why these readings passed despite being beyond the action limits** [illegible]

4. Process Controls

Reviewer's note: Process controls are extensive. Process validation studies demonstrate adequate removal of almost all potential inpurities. The exception is bovine IgG. The bovine IgG issue will be resolved by a commitment to testing future lots and analyzing phase III data.

In-Process Controls Process controls are present at each stage of manufacturing, summarized in the table below:

Stage 1: Preculture and Expansion

Test	Test Method	Specification
Preculture, diluted culture from MWCB:	C	
Cell population density (seeding density)		C
Preculture sampling at every passage, days ' ⟶		
Microscopic examination		
Cell viability		
Cell population density		
Preculture, inoculum:		
Microscopic examination		
Cell viability		
Cell population density		
Bioburden		
Sampling of — inoculum fermenter, at inoculation:		
Cell population density		
Sampling of — inoculum fermenter, at least once per 24 h:		
pH		
Microscopic examination		
Cell viability		
Bioburden		
Cell population density		

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Stage 2: Production Fermentation by Continuous Perfusion

Test	Test Method	Specification
Fermenter sampling, at inoculation:		
Cell population density	C	C
Fermenter sampling, at least once per 24 h:		
pH		
Microscopic examination		
Cell viability		
Cell population density		
IgG content by nephelometry		
Bioburden		
Fermenter sampling at the end of fermentation:		
Mycoplasma		
Cultivable		
Non-cultivable		
Retrovirus testing [e]		
XC plaque assay		
S$^+$L$^-$ focus assay		
Dunni cell assay		
In vitro test for adventitious viruses on:		
Vero cells		
MRC-5 cells		
HeLa cells		
Host C168J cells		
Every harvest container at disconnection from fermenter:		
pH	C	
Microscopic examination		N
Bioburden		
Endotoxin by LAL		

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Stage 3: Direct Product Capture by Protein A Chromatography

Test	Test Method	Specification
Concentrated IgG before freezing:		
pH		
Endotoxin by LAL		
Bioburden		
Protein concentration by OD280		
Identity by IEF		
Purity by GF-HPLC		
Osmolality		

Stage 4: Thawing and Pooling of Direct Product Capture Processed IgG

Test	Test Method	Specification
Thawed eluate pool:		
Endotoxin by LAL		
Bioburden		
Thawed eluate pool after citric acid titration:		
pH		

Stage 5: Solvent Detergent Treatment and Purification by Cation Exchange Chromatography

Test	Test Method	Specification
Solvent detergent treated IgG before dilution:		
pH		
Solvent detergent treated IgG during in-line dilution/column loading:		
pH		
Conductivity		
Cation exchange purified IgG:		
Endotoxin by LAL		
Purity by GF-HPLC		

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Stage 6: Virus Filtration of Cation Exchange Purified IgG

Test	Test Method	Specification
IgG during filtration:		
Protein concentration by OD280		
IgG after filtration and concentration:		
Protein concentration by OD280		

Stage 7: Purification by Primary Anion Exchange Chromatography

Test	Test Method	Specification
Pooled IgG prior to column loading:		
pH		
Conductivity		
Primary anion exchange purified IgG:		
Purity by GF-HPLC		
Endotoxin by LAL		

Stage 8: Purification by Secondary Anion Exchange Chromatography

Test	Test Method	Specification
IgG during column loading:		
Conductivity		
Secondary anion exchange purified IgG:		
Purity by GF-HPLC (individual fractions and pool)		
Endotoxin by LAL		

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Test	Test Method	Specification
cA2 IgG prior to final 0.2 μm filtration:		
Conductivity	C	C
cA2 IgG PFB before freezing:		
Endotoxin by LAL		
Bioburden		
pH		
Protein concentration by OD280		
Immunoreactivity by bioassay		
Purity by SDS-PAGE		
Reduced		
Non-reduced		
GF-HPLC		
Purity		
Identity		

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a If viability falls below — for 3 consecutive days, the fermentation will be terminated.
b If IgG concentration falls below ——— for 3 consecutive days after the fermenter is filled, the fermentation will be terminated.
c Murine retroviruses will be measured on the first 3 fermentation runs when a new Manufacturer's Working Cell Bank (MWCB) is instituted. If no virus is detected, subsequent fermenter runs derived from the same MWCB will not be tested. If virus is detected, testing will continue with the indicated specifications.

Process Validation Process validation studies were performed on the — and the ——— scale process. Three lots of product were examined at the — kg scale, and one lot at the ———scale. One additional ——— scale lot is pending for submission to the BLA as an amendment.

Cell Growth and Harvesting

Fermentation was validated by examining — bioreactor runs (—— days) for cell viability/growth kinetics, IgG production and adventitious agents. In the preculture phase of each run, doubling times ranged from 17 to 48 hours. By about day —of the nine fermentation runs, a plateau level of total cell number was reached; ——— , which held constant until the runs were terminated. The bioreactor run with the maximum number of cumulative generations had — generations. Once plateau cell number was reached (~day —, antibody production remained above 30 pg/cell-day in 7 of the — runs. The two exceptions were Z7B323 and Z6L035. Z6L035 fell below 30 pg/cell-day around day — beyond the time when bioreactor runs are specified to be terminated. Z7B323 barely made it above 30 pg/cell-day, declined to ~20 pg/cell-day, and

was terminated on day ~ This occurred because of a loss of cells when the spin filter clogged and overflowed. Co-cultivation virus testing didn't detect infectious retroviruses in any of the bioreactor runs. TEM detected 7 X 10^8 - 3 X 10^{10} type C particles/ml in harvests from day ~~the~~ highest number was on day ~ beyond the normal termination time of bioreactor runs, and within the validated removal capacity of the purification process.

Antibody purified from early and late phases of fermentation (days ~ was analyzed by bioassay, SDS-PAGE, GF-HPLC, IEF, mass spec, tryptic and oligo saccharide mapping, CD, C-terminal lysine and N-terminal sqxing. No changes during the course of fermentation were evident in the cA2, with the exception of the gradual appearance of slightly elevated levels of de-amidated forms detected by IEF and a decrease in levels of heavy chains with C-terminal lysine (80% at day 14 to 62% at day ~

Purification

Purification was validated by an examination of the removal of chemical and proteins used to manufacture cA2, and by an examination of product at various stages of purification. One ~ scale lot (Z7D117) and three ~ scale lots (5J005, Z6M022, and Z7A286) were analyzed. In almost all cases, data was presented that validated removal below 1-2 ppm. For each potential contaminant, direct measurements in DPC and purified bulk were performed. Additional testing depended on the nature of the contaminant. Several potential contaminants are largely removed by the DPC protein A step, and additional spike/removal studies were performed to demonstrate several logs of removal by subsequent chromatography steps. Many of the potential contaminants are small molecules that would be expected to be diafiltered away in one of the two buffer exchange steps. Only one of the protein contaminants is expected to have an affinity for protein A, bovine IgG. Based on the validation studies of removal of the potential contaminants, only two are potential concerns: bovine IgG and mycophenolic acid.

Contaminant	Direct Measurement	Spike Study
Bovine Serum Albumin	<0.4 ng/mg	
Bovine IgG	0.8 - 14 ng/mg	-
Host Cell DNA	< 0.7 pg/mg	
EDTA	< 0.15 mM	-
Pluronic F68	< 4.6 µg/mL	-
Excyte	-	
Bovine Transferrin	0.2 - 1.2 ng/mg	-
Bovine Insulin	< 0.019 ng/mg	
Mycophenolic Acid	< 15.5 ng/mL	-
Hypoxanthine	< 4.6 ng/mL	-
Xanthine	< 4.2 ng/mL	-
Hydrocortisone	< 0.3 ng/mL	-
Tween 80	< 20 µg/mL	-
Tri-n-Butyl phosphate	< 0.5 µg/mL	-
Protein A	< 125 ng/mg	
Guanidine	< 5.3 µg/mL	
Heavy Metals	In control	-
Bacterial Endotoxin	In control	-
Bioburden	In control	-
Virus	-	
Yield	74.3 ± 17.2%	-

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Note: Bovine IgG levels in three lots of [redacted] scale PFB were 0.8, 2.0 and 2.4 ppm, while 14 ppm was present in one [redacted] manufacturing scale lot (questions 1 & 2):

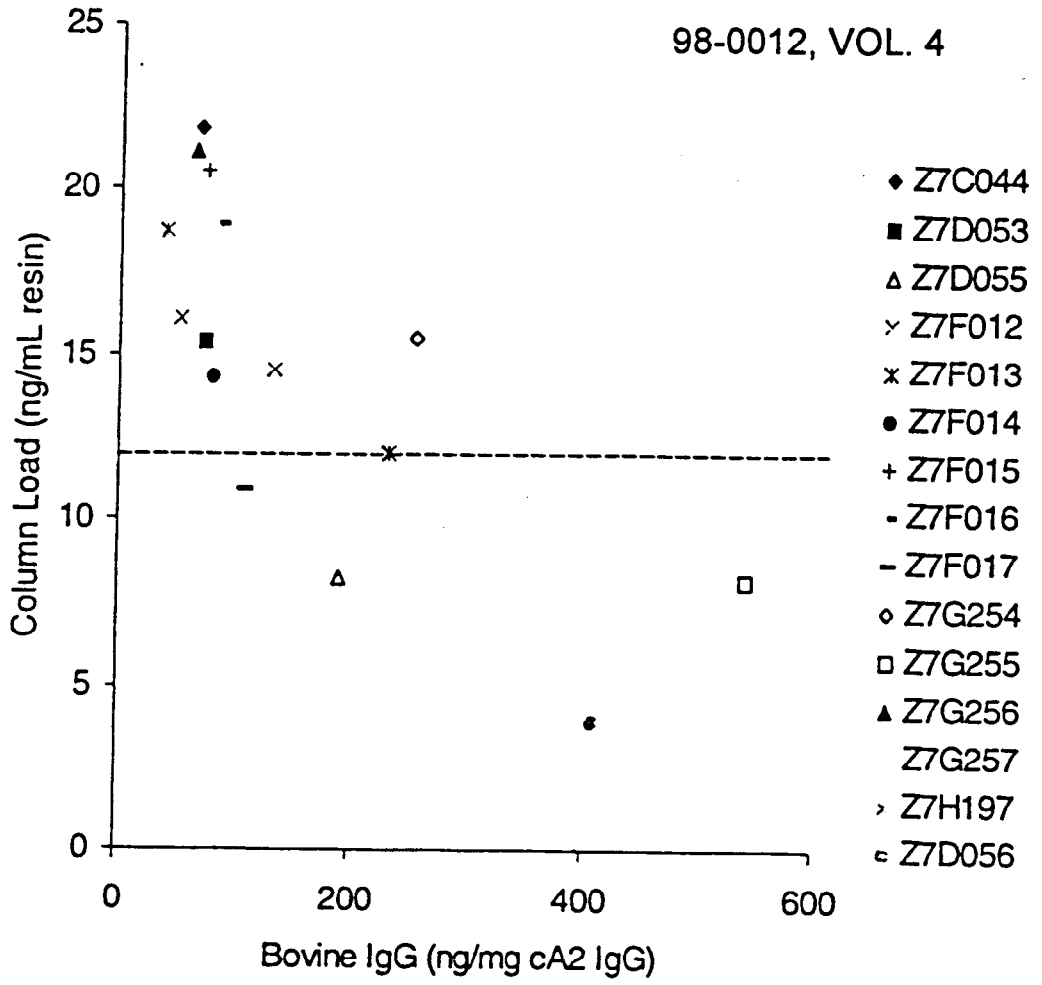
Bovine IgG levels in process intermediates (per mg cA2 IgG) 98-0012, VOL. 4

Lot Number	CCS/ Harvest (µg)	Protein A IgG (ng)	Cation Exchange IgG (ng)	Primary Anion Exchange IgG (Q1) (ng)	Secondary Anion Exchange IgG (Q2)[a] (ng)
5J005	0.8	15	9.1	7	2.0
Z6M022	0.9	18	10.2	10	2.4
Z7A286	0.6	6.9	2.9	2.7	0.8
Z7D117	NT[b]	102[c]	87	87	14

[a] Sample analyzed was PFB since the cA2 IgG concentration was higher than the Q2 intermediate.
[b] Bovine IgG levels in all harvests were not tested. Data for representative harvests are shown in Table 106.
[c] [redacted] pool tested prior to S/D treatment.

Bovine IgG is a contaminant in BSA (Cohn fraction V) used to formulate media, and is carried through into the product. Two steps in manufacturing that remove substantial amounts are protein A and the second anion exchange column. The higher levels in [redacted] kg scale cA2 probably result from direct product capture

(DPC) protein A chromatography. In the ___ scale lots, there was a concentration step before protein A, allowing for the column to be loaded closer to capacity. Centocor has data that shows that loading the column nearer capacity results in less bovine IgG in the product:



Centocor's strategy to minimize bovine IgG is to adopt a <400 ng IgG/mg BSA acceptance criteria for the BSA used in fermentation and to load the DPC protein ~~column~~ They calculate that given a >1.9 log reduction of bovine IgG by the manufacturing process, they will have at most 25 ppm bovine IgG in the product.

Given that cA2 is

Bovine IgG assay results for Ca2 IgG FVP used in clinical trials 98-0012, VOL. 4

Sample ID	cA2 IgG Concentration (mg/mL)	Clinical Study	Bovine IgG Concentration (ng/mL)	Bovine IgG Concentration (ng/mg Ca2)

Mycophenolic acid is a poison that is used for selection of *gpt* expressing C168J transfectants. They have demonstrated that mycophenolic acid levels are below their threshold detection limit of ——— in DPC and purified bulk. No spike/removal studies were performed on any other purification step.

Process intermediates from each of the four validation lots were examined by SDS-PAGE, IEF and GF-HPLC to demonstrate the function of the processing steps. Side-by-side comparisons of process intermediates revealed that intermediates from all lots were similar biochemically, that the product didn't degrade during processing, and that the purity of cA2 increased during processing. The overall yield of the process ranged from ——— in the four lots.

Microbiology

Virus removal validation was performed on the manufacturing process using 3 model viruses: The steps that were validated for virus removal were solvent/detergent, protein A chromatography, anion exchange and ——— filtration. Both the scale were validated. Validation of ——— filtration was performed with ———, the worst case scenario virus for filtration because of particle size.

They validated with [illegible] because the shear forces by recirculation of solution over the filter destroy [illegible], making accurate measurement of the sieving effect meaningless. Data of [illegible] removal by the anion exchange steps was not collected for the [illegible] scale because [illegible] binds tightly to the resin and can only stripped off by [illegible] They claim that this inherent property of the resin doesn't change with the scale and they don't need to revalidate.

Virus log removal data from the 8 kg scale:

Step	[illegible]	[illegible]	[illegible]
DPC protein A	>6.1	2.8	3.7
Solvent/detergent	>3.0		
Anion exchange, primary	(>4.3)		
Anion exchange, secondary	(>5.7)	>6.5	
	2.5	2.5	2.5
Total	>11.6	>11.8	6.2

A validation study was performed on cleaning solutions used for sanitizing columns and diafiltration devices.

Stationary equipment sterilization is accomplished by steam-in-place (SIP).

Sample	Specification
— ermenter (sampled at least once per 24 h) (Stage 1)	Bioburden <
— production fermenter (sampled at least once per 24 h) (Stage 2)	Bioburden
Harvests (at disconnection from fermenter) (Stage 2)	Endotoxin Bioburden
Protein A purified and concentrated cA2 (prior to freezing) (Stage 3)	Endotoxin Bioburden
Thawed, pooled Direct Product Capture eluates (Stage 4)	Endotoxin Bioburden
Cation exchange purified cA2 (Stage 5)	Endotoxin
Viresolve system retentate and filtrate (Stage 6)	Endotoxin Bioburden
Primary anion exchange column effluent (Stage 7)	Endotoxin
Primary anion exchange purified cA2 (Stage 7)	Endotoxin
Secondary anion exchange column effluent (Stage 8)	Endotoxin
Secondary anion exchange purified cA2 (Stage 8)	Endotoxin
Ultrafiltration system retentates (Stage 9)	Endotoxin
Concentrated cA2 PFB (prior to freezing) (Stage 9)	Endotoxin Bioburden

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Bioburden levels from each production stage were reported from the four validation lots. Bioburden was within specifications in almost all cases. The exceptions were mold present in ..... filters before use (1-14 cfu/ml) and in DPC harvests from lot Z7A294-H03 (80-99 cfu/ml before UF, TNTC after). After a sanitization of the column, the problem abated but bioburden was still detectable. **Note: it should be determined whether that particular harvest was discarded and whether an investigation was performed on how these contamination's were introduced. The genus (and species if possible) of both the bacteria and the mold should be identified**
Both high bioburden incidents were handled during the inspection, see EIR. Endotoxin levels were below detection in several in process stages in these lots.

Monitoring of air and surfaces occurs on a periodic basis. Viable particles are quantitated by air sampling, agar contact (surfaces) and settling plates. Door, floor, wall and bench surfaces are monitored. Airborne particles are detected by a calibrated particle counter. Warning and action limits are set depending on room classification; SOPs are in place for sterilization and bioburden control. Representative data of one year of monitoring room —

is presented in the BLA. Moving averages of environmental bioburden of these rooms were within action limits, except for room [redacted] where settle plate results indicate that high levels of airborne bioburden were present from March 1997 until August 1997. **Note: It should be determined whether an investigation was performed to find the source of the airborne bioburden. The genus (and species if possible) of the bioburden should be identified ([redacted]). The issue of environmental monitoring at Centocor BV was looked into by [redacted] during the inspection.**

5. Reference Standard(s)

A three tiered reference standard system is used for cA2; research, master and working. There are [redacted] research reference standards: [redacted] were produced by the first transfectant [redacted] are liquid formulations prepared from the current transfectant [redacted] on 12/27/91 and 5/7/92 and stored at < -60°C. These lots were qualified by comparison to OD_{280}, pH, DRID, SDS-PAGE, GF-HPLC, ELISA, color and particle data from the earlier [redacted] research lots.

The master (primary) reference standard is lot [redacted], derived from bulk lot 5J005 produced 9/26/96. This lot is in liquid formulation and is stored at [redacted] vials of this lot are designated as master reference standards and [redacted] vials are designated as working reference standard lot [redacted]. This lot was qualified by comparison to research reference lot 92F02AB. The lot was analyzed by OD_{280}, bioassay, pH, DRID, SDS-PAGE, IEF, GF-HPLC, ELISA, color, turbidity, bioburden, peptide mapping, oligo saccharide mapping, mass spec, analytical ultracentrifugation, C-terminal lysine content and visible particles. The master and working reference standards will be requalified yearly against current production and master lots.

6. Specifications / Analytical Methods

Drug Substance Specifications And Tests

Specifications And Analytical Methods

PFB tests and specifications are:

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Preformulated Bulk (PFB) Test	Test Method	Specification
Endotoxin by LAL		
pH		
Protein concentration by OD280		
Immunoreactivity by bioassay		
Purity by SDS-PAGE Reduced		
Nonreduced		
GF-HPLC Purity		
Identity		
Bioburden		

- LAL is a chromogenic substrate based method. It was validated by comparing rabbit pyrogen results on 6 FLP lots with LAL results (Guideline for LAL, 1987).
- The pH is read by a pH meter calibrated to NIST traceable standards (USP 23 <791>).
- Protein concentration is measured with a NIST traceable metal-on-glass filter calibrated spectrophotometer. The cA2 extinction coefficient is [redacted] cm. The assay was validated for linearity [redacted], accuracy, repeatability, intermediate precision and specificity.
- Bioassay is a [redacted] 164 growth inhibition assay. TNF inhibits their growth, cA2 neutralizes TNF. [redacted] growth is read by MTT metabolization. The

assay was validated for linearity (60-130%), accuracy, repeatability, intermediate precision, range and specificity.

- SDS-PAGE gels are run reduced and non-reduced on 8-18% linear gradient gels. The gels were validated for repeatability, intermediate precision and LOD (0.02%)
- GF-HPLC is a run using a < column. The column was validated for accuracy, specificity, linearity, LOD (0.1% aggregate) and LOQ, repeatability and intermediate precision.
- Bioburden testing is performed by filtering 1 ml of cA2 and overlaying the filter on soybean casein digest media plates. The assay was validated by testing the filters for bacteriostasis and fungistasis.

- **Note: Because of the inherent variability of the bioassay, the anti-TNF ELISA that they has performed on earlier lots should be included as a lot release assay. The assay should be validated .**

- **Note: An assay for bovine IgG should be included for lot release and upper limit specifications should be set. The assay should be validated**

Certificates Of Analysis And Analytical Results

Three lots of cale (Z6M022, Z7A286, Z7D117) and 1 lot of scale (Z7H382) cA2 were tested and found to be within specifications:

PFB Lot	5J005[a]	Z6M022	Z7A286	Z7D117	Z7H382
Release Tests					
Preformulated Bulk (PFB)					
LAL (EU/mg)	< 0.04[b]	< 0.02	< 0.02	< 0.02	< 0.02
pH	7.1	7.2	7.1	7.1	7.2
Protein concentration (mg/mL)	20[c]	42	45	48	45
Bioassay	89%	89%	92%	99%	97%
SDS-PAGE					
Red: purity	98.6%	97.3%	98.0%	98.6%	98.1%
new bands	none > 0.1%	none > 0.1%	none > 0.1%	none > 0.1%	none > 0.1%
electrophoretic profile	CFS[d]	CFS	CFS	CFS	CFS
Nonred: ratio IgG band	1.0	1.0	0.9	1.0	1.0
new bands	none > 0.1%	none > 0.1%	none > 0.1%	none > 0.1%	none > 0.1%
electrophoretic profile	CFS	CFS	CFS	CFS	CFS
GF-HPLC					
purity	99.6%	> 99.9%	> 99.9%	> 99.9%	> 99.9%
identity, retention time difference (min)	0.0	0.0	0.0	0.0	0.0
Bioburden	0 cfu/mL	0 cfu/mL	0 cfu/mL	0 cfu/mL	0 cfu/mL

[a] Lot formulated to 18-23 mg/mL.
[b] Specification for first lot:
[c] Specification for first lot:
[d] CFS, conforms to Reference Standard.

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Impurities Profile

Purified bulk was analyzed for contaminant proteins by SDS-PAGE and western blotting. Validation of the removal of media derived contaminants (BSA, bovine IgG, etc.) was performed separately, so the impurities profile analysis focused on examining host cell contaminants and degradation products. Silver stained SDS-PAGE gels have a number of minor bands besides the main cA2 bands and the impurities profile analysis attempted to identify each.

Host cell and media proteins were looked for by western blotting. Three antisera were produced from a rabbits immunized with protein A purified culture supernatant from mock transfected [redacted] cells and whole bovine serum and goats with normal mouse serum. The anti-bovine serum was absorbed with donkey, mouse and human sera. Western blots were prepared cA2 final product and appropriate positive and negative controls. No contaminant bands were detected in these western blots, except possibly bovine IgG (present at ppm levels). The blots had some background; most of the antisera bound to cA2. **Note: [redacted] cell paste should have been used to immunize rabbits, as protein A purified culture supernatants probably don't contain internal cellular proteins that may be introduced into the product if there is a large amount of cell death in the bioreactors.**

7. Container/Closure System

The drug substance is held and shipped in 10 or 20 L Nalgene polycarbonate containers. Closures are polypropylene screw closures with thermoplastic elastomer gaskets. Storage is at -40°C. Letters of cross-reference to DMF 7325 ([redacted]) and DMF 1562 ([redacted]). **Note: this system is adequate.**

8. Drug Substance Stability

Drug substance stability studies have been initiated only recently because the manufacturing process has established recently. They will study the stability of culture media, harvest, DPC, downstream process intermediates, and PFB. **Note: this plan is adequate. but updated information should be submitted prior to licensure ——. The dating period of intermediates will be no longer than 6 months beyond real time data as agreed to during the pre-BLA meeting.** Storage conditions are summarized below:

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	Storage Temperature	Proposed Shelf Life
Serum-free Media	8 - 14°C	
cA2 IgG Harvest	8 - 14°C	
Downstream Purification Intermediates:	2 - 8°C	
Stages 5 and 6		
Stages 7 and 8		
Frozen Direct Product Capture cA2 IgG (DPC)	-40°C	
Frozen Preformulated Bulk (PFB)	-40°C -25°C	

The ability of shelf-life stored (—— ;) and ——) media was evaluated for its ability to support growth and IgG secretion of —— cells. No difference in growth rates or IgG secretion from cells in fresh media was apparent.

Stability studies of harvests are ongoing. They will test 500 ml aliquots from 3 harvests at 0, 2, 4, and 8 weeks of storage. At each point they will make DPC and test it by IEF, SDS-PAGE and GF-HPLC to look for cleavage, deamidation or other degradation. They also plan to stability test DPC, PFB and FLP derived from 4 week old harvest.

Holding times for downstream process intermediates were set based on manufacturing experience and the need to minimize bioburden. The hold times are short ——. They don't plan on performing stability tests, rather they will rely on in process, release and stability testing to show lot-to-lot consistency.

DPC stability will be studied in real time and by an examination of thermal properties. To analyze whether the product is completely immobilized in a frozen matrix at -40°C, an electrical resistance analysis was conducted at ' —— When complete freezing occurs, electrical resistance is high. Their analysis revealed that the collapse temperature of the DPC, where mobility of water is near zero, is -34°C while the temperature where resistance plateaus because the mobility of all components of the matrix is near

zero is -40°C. Thus, -40°C is a temperature where the DPC is completely frozen, limiting the potential of chemical and physical damage of the cA2. The stability of DPC at Two lots of stored product will be analyzed by OD_{280}, GF-HPLC, IEF and bioassay. DPC stored for 3 years will be purified and formulated into FLP for stability analysis

DRUG PRODUCT

1. Composition

Note: The composition is adequate.

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TO BE
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RELEASABLE

5. Process Controls

Note: process controls are adequate unless otherwise noted.

In-Process Controls

The following process controls apply to drug product manufacture: -

Test	Test Method	Specification
	Frozen Preformulated Bulk (PFB)	
Identity by GF-HPLC		
	Formulation Buffer Before Filtration	
pH		
Osmolality		
Endotoxin Content		
Bioburden		
	Pooled PFB Before Filtration	
Endotoxin Content		
Bioburden		
	PFB After Filtration	
Protein Concentration		
	Final Bulk (FB) Before Filtration	
Protein Concentration		
Endotoxin Content		
Bioburden		
	Final Vialed Product Before Lyophilization	
Bulk Sterility		No growth
Fill Volume		
	Final Lyophilized Product (FLP)	
Sterility		No growth
Manual Inspection (100%)		Product Defects: No discoloration of cake, no foreign particulate matter, no meltback, no liquid, no other foreign materials, and acceptable fill volume/cake height. Container/Closure Defects: No cracks or broken glass, no missing components, no glass imperfections, no closure or component defects.

[a] The volume tested is equal to 1 mL for each liter of PFB pooled.

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- GF-HPLC is performed using the same SOP as the validated GF-HPLC at Centocor.
- pH is performed with NIST traceable standards
- Osmolality is performed using a OSMETTE Model 2007
- LAL is performed according to USP 23 <85>.
- Bioburden is measured by membrane filtration.
- Protein concentration is measured by A_{280}. A comparison was performed between measurements at ——— and Centocor and were found to be equivalent.
- Sterility is performed according to 21 CFR 610.12
- Fill volume determination is performed by a computerized fill volume monitoring system.
- Manual visual inspection is performed on 100% of the vials by trained operators.

Process Validation

6. Specifications & Test Methods For Drug Product

Note: Specifications and test methods are adequate unless otherwise noted.

Sampling Procedures

Vials are selected randomly for release testing. — vials are tested for sterility and uniformity of dosage form. — vials are tested for residual moisture. — is tested for reconstitution time and turbidity. — vials are tested for visible particles. — vial is reconstituted for testing by LAL, pH, A_{280}, bioassay, DRID, SDS-PAGE, IEF, and GF-HPLC.

Specifications & Methods

There are two sets of drug product specifications: release and product. The separate, less stringent, product specification applies to stability testing where some product degradation (e.g. accumulation of visible particles) is expected to occur. Each of the 5 FLP lots lyophilized at C were within release specifications. The product specifications are:

Fermentation Samples (at end of fermentation)

Test	Test Method	Specification
Mycoplasma		
Cultivable		
Non-cultivable		
Retrovirus testing [a]		
XC plaque assay		
S+L- focus assay		
Dunni cell assay		
In Vitro test for adventitious viruses on:		
Vero cells		
MRC-5		
HeLa cells		
Host C168J cells		

Final Bulk

Test	Test Method	Specification
Sterility	21 CFR 610.12 USP <71>	

Final Lyophilized Product

Test	Test Method	Specification
Sterility	21 CFR 610.12 USP <71>	C
Endotoxin by LAL		
Appearance		
Color after reconstitution		
Visible particles after reconstitution		

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Turbidity after reconstitution		
Residual moisture		

Final Lyophilized Product

Test	Test Method	Specification
Reconstitution time		
pH		
Protein content by OD280		
Uniformity of dosage units		
Immunoreactivity by bioassay		
Identity by DRID		
Purity by SDS-PAGE Reduced		
Non-reduced		
GF-HPLC Purity		
Identity		
Charge heterogeneity and identity by IEF		

[a] — .etroviruses will be measured on the first— .ermentation runs when a new Manufacturer's Working Cell Bank (MWCB) is instituted. If no virus is detected, subsequent fermenter runs derived from the same MWCB will not be tested. If virus is detected, testing will continue with the indicated specifications.

[b] Brackets indicate initial release specifications.

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- SDS-PAGE, GF-HPLC, pH, LAL, IEF, protein concentration, bioassay are the same as those used to test purified bulk.
- Sterility is performed according to 21 CFR 610.12.
- Appearance is performed on 100% of vials at ——— using their SOP.
- Color is performed according to USP <631> with color standard A as a reference.

- Visible particles is performed using at least two trained operators by comparison to suspensions of 66 µm fluorescent polymer microspheres in WFI. Six particle standards are used:

Standard	Particles/10ml
A	0
B	25
C	50
D	100
E	200
F	400

- Turbidity is measured with a [redacted] turbidimeter and [redacted] and formazine standards. Results are converted from nephelometric turbidity units (NTU) to opalescence categories of EP V.6.1. The assay was validated for linearity between 2.5-1000 NTU.
- Residual moisture is measured using Karl Fischer coulometric assay. The assay was validated for linearity between 0.04-2.4% moisture.
- Reconstitution time is measured by visual observation.
- Uniformity of dosage is performed to comply with USP <905>.
- DRID is an Ouchterloney assay. It was validated as having a reaction pattern specific for cA2; ReoPro and Myoscint yield different pattern.

7. Container/Closure System

Note: The container closure system is adequate.

Vials are 20 ml USP type 1 borosilicate glass vials from [redacted]. Letters of DMF cross-reference are present in the BLA. Vials are inspected at [redacted] upon arrival for critical, major (A & B) and minor defects using Military Standard 105E level II procedures.

Stoppers are 20 mm [redacted] gray flanged S-87-J lyophilization stoppers (DMF 1546). The stoppers comply with physicochemical extraction characteristics in USP <381> and biological reactivity characteristics in USP <88>. A letter of DMF cross-reference is present in the BLA. The stoppers are inspected at [redacted] upon arrival for critical, major (A & B) and minor defects using Military Standard 105E level II procedures.

Flip-off caps are lacquered aluminum with a polypropylene flip-off button [redacted] The caps are inspected at [redacted] upon arrival for critical, major (A & B) and minor defects using Military Standard 105E level II procedures.

Closure integrity is monitored at [redacted], on each vial as it is sealed by a [redacted] rotary capper/seal force monitor III. A media fill of 10,000 vials was

performed as well. None of the media fill vials became contaminated or failed a dye intrusion test. Thus, there is at least a 95% confidence that < 0.1% of the vials have inadequate closures.

8. Microbiology

Note: microbiology control is adequate.

All sterile operations occur within building [illegible] in areas designated as class [illegible] Storage of sterile equipment and non-sterile bulk manipulations occur in class [illegible] areas. Filtration of the formulated bulk through [illegible] filters is considered to be the start of sterile operations. Filters are tested routinely using a [illegible] filter integrity tester. Sterilization information is kept in the LUMAC log of the batch records.

Critical processes:

- Transfer of sterilized components to filling area. Transfer occurs via a UV pass through.
- Aseptic assembly. The final assembly of the filling pumps occurs in aseptic filling area. Vials enter depyrogenated and stoppers are fed into the stopper bowl from autoclave bags under full ceiling HEPA filters.
- Isolation system. Each room with sterile operations has ceiling HEPA filters over work areas. The lyophilization rooms have full ceiling HEPA filters.

Equipment for sterilization of equipment and components are:

- Sterilizer F-39, Room [illegible] (steam and ethylene oxide)
- Sterilizer MS-9, room [illegible] (steam only)
- Tank steam stations, rooms [illegible]
- [illegible] depyrogenation) Tunnels model TLQ B05, rooms [illegible]
- UV airlocks (pass through into sterile areas)

Sterilization of the following equipment and components has been validated using spore strips and readings from thermocouples:

- Filtration cartridges and assemblies
- Holding vessels
- Filling equipment
- Vials, also validated for endotoxin removal by depyrogenation tunnel (6.0 logs).
- Stoppers, wash also validated for endotoxin removal (6.0 logs).
- Freeze dryers, sterilized by ethylene oxide were validated by the ETO biological challenge test (B. subtilis). The absence of ethylene oxide residuals was also validated.

Media fills each calendar quarter. The action limit of media fills are a contamination rate of 0.1% with 95% confidence (4 vials/10,000). The alert limit is 1 vial/10,000. Since 1996, media fills were performed on line and on line No media fills exceed the action limit, but one on line and two on line exceeded the alert limits. Procedures are in place if a media fill fails.

Environmental monitoring tests for air viables (settling plates, Reuter centrifugal air sampler), air non-viables (Climet particle counters), surface viables (RODAC contact plates), and personnel viables (finger impressions and body RODAC). Monitoring is at least once per shift (3/day) in rooms (line and (line . Action limits are:

Test	*Sites tested*	*Action limit*
Contact plate	12	
Settling plate	6	
RCS	1	
Climet	1	
Finger	2	
Body RODAC	4	

Since 1996, line one has failed 10 of 7588 RODAC tests, 5 of 4980 RCS tests and 0 of 7313 plate exposure tests. Line 9 has failed 5 of 5443 RODAC tests, 1 or 2943 RCS tests, and 0 of 4341 plate exposure tests. Procedures are in place for environmental investigation when action limits are exceeded.

9. Drug Product Stability

Note: Centocor has initially proposed storage at This was revised to 2-8°C based on stability data (attachment 6). CBER agreed with Centocor to allow an 18 month expiration date if they have 12 months of adequate real time stability data (see minutes of 1/98 pre-BLA meeting). The final update of this data will be submitted in June.

The initial proposed storage condition of Infliximab is Stability testing on lots 95K06 and 96E06 scale Lyo I) have extended for 78 and 52 weeks. Stability testing on lots 97A07, 97A10, 97C07 (scale, Lyo II), 97E08 and 97E09 (scale, Lyo II) have extended 26 (scale lots) and 13 (scale lots) weeks. The vials are tested for appearance, reconstitution time, visible & subvisible particles, turbidity, residual moisture, protein content, bioassay, SDS-PAGE, GF-HPLC, IEF, pH and sterility. So far, all vials of cA2 held at are within specifications. Vials of cA2 held at is also within specifications, but a trend towards the accumulation of visible and subvisible particles is evident. This trend is not evident in cA2 held at and doesn't

correlate with a loss of bioactivity. Newer lots have less residual moisture, this may effect the formation of particles.

Note: The key study using cA2 from — scale lots has extended only a short period (13 weeks). Centocor wants a —— expiration date, but agreed to 18 months pending updated data submitted as an amendment to the BLA (——).

Photostability studies have concluded that cA2 is photolabile when illuminated under extreme conditions (5 X 1.2 million lux hours with UV energy > 200 watt hours/m^2) with purity by SDS-PAGE and visible particles stability indicating. Illumination under less extreme conditions (1.2 million lux hours with UV energy > 200 watt hours/m^2) didn't cause a noticeable deviation from specifications, so there will be no precaution against exposure to light in the labeling.

Reconstituted stability studies were performed for 24 hours using cA2 held at room temperature in glass bottles and infusion bags (polyethylene, ethyl vinyl acetate, polyvinyl chloride). The cA2 was stable in glass bottles and polyethylene and ethyl vinyl acetate infusion bags. PVC bags were found to be incompatible with cA2 because polysorbate 80 in cA2 causes di(2-ethyl-hexyl)phthalate (DEHP) to leach from the bags into the infusion solution. Labeling statements reflect this. **Note: during labeling review, check to see if wording is strong enough (see 6/15/98 labeling meeting minutes).** The ability of in-line filters to clear visible and subvisible particles from stressed (shaken) cA2 was tested. Supor PES 1.2µm filters —— cleared almost all visible (>F vs. A) and subvisible particles (1000-7000 vs. 25-173 particles > 10µm) without a discernible loss of protein content. Non-DHEP PVC infusion lines were found to be compatible with cA2.

OTHER CONSIDERATIONS

1. Investigational product/formulation

2. Environmental assessment

Centocor is claiming categorical exclusion under 21 CFR 25.31(c).

3. Method validation

The following release assays were validated. **Note: The validations were adequate except for the visible particle assay.** Validation reports provided in the submission:

- pH (SOP 103X). pH meters are calibrated daily against NIST traceable standards as described in USP <791>.
- Protein concentration (SOP 003A). Protein concentration is measured by UV spectrophotometry. The method was validated (report ASV-9502.P) for linearity (———), accuracy (recoveries ——— of theoretical

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Comments for the sponsor (note: 1, 2, and 3 were conveyed in a February telecon, the rest in a letter dated 4/15/98 or on inspection).

1. Serum samples should be assayed for antibodies to bovine IgG. If immunogenic levels of bovine IgG were present in lots of cA2, it may be possible to detect anti-bovine IgG antibodies in patient sera (assuming that they mounted Th2 responses). If patients produced anti-bovine IgG antibodies, they should be analyzed for allergic type adverse reactions. **(resolved,** [redacted]

2. The early trials used cA2 lots with high levels of bovine IgG (up to [redacted] ppm). Adverse event rates should be examined in these early trials to see if allergic type adverse events occurred and if their incidence correlated with bovine IgG levels. **(resolved,** [redacted]

3. The virus removal capacity of the anion exchange columns of the [redacted] scale process should be revalidated. Virus removal data for this step from the [redacted] scale was provided. However, this data is insufficient for validation of the [redacted] kg scale process because there was an increase in column loading. **(resolved,** [redacted]

4. Please submit SDS-PAGE and GF-HPLC data from the qualifying lot Z7H382 when it is available. **(resolved,** [redacted]

5. In GF-HPLC assay results from qualifying lots, the product is consistently > 99.9% pure, while SDS-PAGE analysis detect impurities of up to 2.0%. Please explain the discrepancy between the two assays. **(resolved,** [redacted]

6. Please describe the manufacture of [redacted] In particular, are there heat inactivation or autoclaving steps? Please describe steps designed to control porcine parvovirus. **(resolved,** [redacted]

7. In the cell line genetic stability studies, sequencing of RT/PCR products was performed to detect potential aberrant transcripts. However, the sensitivity of the assay was 25%. It should be re-performed with an assay with a higher sensitivity. Please comment. **(resolved,** [redacted]

8. The [redacted] batch records indicate that vials of Infliximab FLP are labeled with lot numbers that do not correspond with the FLP lot number. For example, [redacted]

[redacted] Please comment. **(resolved during inspection).**

9. At step —, of the batch records for DPC lots Z7G256, Z7C044, and Z7D053 GF-HPLC purity is listed as 100%, while more than one band is evident in the actual GF-HPLC trace. If late peaks are ignored because they are considered to be buffer or other irrelevant peaks, there should be a clear description in the batch record and the SOP of how to identify peaks that should be disregarded and how to calculate % purity based on GF-HPLC traces. **(resolved,** ——————— **,**

10. A breakdown of what equipment at . . ——— s dedicated to cA2 should be provided. Form ⊂ ⊃. attached to operational equipment. This form contains a record of previous use and in some instances it is unreadable because the ink used to record information smears after autoclaving. There are also instances where equipment is shared with other processes may be product contact equipment. For example, are a "short needle bar (1.2)" previously used to process ⊂ ⊃ (pg. 105 of 124 of the batch record); a "transfer hose" and a "20 L bottle" used to process ⊂ ⊃ (pg. 111 of 124); and a "res head" used to process ⊂ ⊃ (pg 52) dedicated to cA2? Are these items product contact equipment? **(resolved, see attachment 6)**

11. In some instances in the ⊂ ⊃ batch records, action limits of environmental monitoring were exceeded but still were recorded as "passed". RODAC readings on 5-30-97 from floor A of room ⊂ ⊃ had 18 cfu, but was "satisfactory" (action limit is — cfu/plate). Personnel readings from ⊂ ⊃ right hand on 6-5-97 were 28 cfu, but was scored as "pass" (action limit is ⊂ ⊃ cfu/hand). What actions were taken in these two cases? **(resolved, see** ⊂ ⊃

12. Mycophenolic acid is used to maintain selection of *gpt* expressing ⊂ ⊃ transfectants during fermentation. Mycophenolic acid levels have been demonstrated to be below the assay detection limit of 16 ng/ml in DPC and purified bulk. However, mycophenolic acid is a poison so reduction below 16 ng/ml should be demonstrated. Removal validation studies should be performed on the downstream chromatography steps to assure that the maximum potential contamination of mycophenolic acid in Infliximab is extremely low. **(resolved, see attachment 6)**

13. Mold contamination was detected in ⊂ ⊃ filters before use ⊂ ⊃ fu/ml). Was the genus (and species if possible) of the mold contamination identified? **(resolved during inspection and by telecon, see EIR,** — ⊃

14. Environmental monitoring of room — of Centocor BV revealed that high levels of airborne bioburden was present between 3/97 and 8/97. Was an investigation performed to find the source of the airborne bioburden? Was the genus (and species if possible) identified? **(Resolved, T.A. investigated the envrionmental monitoring of Centocor BV during the inspection).**

15. DPC lot Z7C040 had bioburden levels that exceeded in process specifications (TNTC cfu/ml after ultrafiltration). Was this DPC lot discarded? **(resolved,**

16. bioassays have inherent variability. In investigational lots a TNF binding ELISA was used as a release assay. As ELISA assays have less inherent variability, the TNF binding ELISA should be included for lot release to supplement the bioassay. A validation study of the TNF binding ELISA should be performed. Please comment. **(resolved,**

17. An assay to determine levels of bovine IgG in FLP or PFB should be performed for lot release. A reasonable upper limit specification for bovine IgG should be set. A validation study of the bovine IgG assay should be performed. Please comment. **(resolved,**

18. Updated stability data for harvests, DPC lots Z7D055 and Z7F012; FLP lots 98E08 and 98E09 and PFB lots Z7D117 and ZH382 should be submitted as an amendment to the BLA. Current FLP stability data does not support a dating period. Please describe plans for stability testing future scale FLP lots. We recommend that at least 1 FLP lot be placed on stability per year. **(resolved,**

19. The upper limit specification for moisture in FLP should be lowered based on manufacturing experience. Please comment. **(resolved,**

20.

21. Comparability studies performed on investigational product included comparisons of lots 94D02, 94L02, 95K06 and 97A07. Each of these lots was made with the [illegible] scale process. The comparability studies should include side-by-side comparisons with a [illegible] scale process lot. Please comment. **(resolved, see attachment 6)**

22. The visible particle assay compares reconstituted cA2 to standards comprised of 66 mm polystyrene beads in WFI (A through F). The method was validated by comparing results from 3 operators who categorized 30 bead suspensions and 18 heat stressed cA2 FLP vials. The test articles were made to resemble product with 25, 100 and 400 particles/vial. In the validation report (PDVR97015), the operators consistently split the 25 particle standard between B and C and the 100 particle standard between D and E, as expected. However, the 400 particle standards were not evenly split between F and >F, rather they were almost always placed in category F. Thus, the operators appeared to have a bias against failing test articles. The assay should be redesigned. It should have at least one standard exceeding product specifications, G with 600 or 800 particles/vial. The assay should be performed with blinded positive and negative controls scored concurrently with test article. The positive and negative controls should bead or cA2 suspensions that fall into categories A-G and >G. The revised assay should be revalidated. **(resolved,** [illegible]

23. SOP103X, [illegible] describes the operation of the [illegible] The SOP is comprised of three parts; [illegible]. The final section or [illegible] was written in a manner that appears difficult for the average operator to follow. It should be re-written with an emphasis on clarity. It should provide a step by step explanation of how to design, implement, and test code on the machine. Please comment. **(resolved,** [illegible]

24. An anti-C116E anti-Id control should be added to the DRID assay to detect potential product contamination with C116E antibody. Please comment. **(resolved,** [illegible]